Effect of Irradiation on the Inhibition of *Clostridium botulinum* Toxin Production and the Microbial Flora in Bacon

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ABSTRACT

The effect of irradiation (0.5, 1.0, 1.5 Mrad) on the microbiological safety and stability of temperature-abused (27°C, 60 days) bacon cured with 1.5% NaCl, 0.25% sucrose, 0.3% $Na_5P_3O_{10}$, 0.055% Na-erythorbate, with or without $NaNO_2$ (40 μg/g) was determined. Unioculated bacon (120 μg $NaNO_2$/g) had a sour odor within 13 days, and, if inoculated with ca. 2 *C. botulinum* spores/g, 73% of the samples became toxic within 60 days. Irradiation with 0.5 Mrad prevented spoilage of uninoculated bacon by virtue of reducing the aerobic plate count to <1.0/g; irradiation with 1.0 Mrad sterilized bacon. Pouches of bacon, inoculated with *C. botulinum* spores (2 spores/g), swelled and became toxic; development of swelling and toxicity was delayed by incorporation of $NaNO_2$ (40 μg/g) or by irradiation with 0.5 Mrad. Irradiation with 1.0 Mrad decreased the number of swollen and toxic pouches. Irradiation with 1.5 Mrad prevented swelling and toxicity of bacon inoculated with 2 spores/g.

INTRODUCTION

SODIUM NITRITE ($NaNO_2$) is incorporated in a wide variety of meat products to obtain characteristic color (Cassens et al., 1979) and possibly flavor (Cho and Bratzler, 1970; Wasserman and Talley, 1972), as an antioxidant to help prevent off-odors and flavors (Cho and Bratzler, 1970), and to contribute toward the inhibition of foodborne spoilage and pathogenic microorganisms, especially toxin formation by *Clostridium botulinum* (Pivnick et al., 1970). Under certain circumstances (e.g., frying of bacon) the nitrite may react with amines to produce carcinogenic nitrosamines (Crosby and Sawyer, 1976; Gray and Randall, 1979; Preussmann, 1974; Sander, 1974). Certain changes in processing have been made, including: elimination of nitrate where possible and the addition of ascorbate or isoascorbate (erythorbate) to diminish nitrosamine formation by enhancing the reduction of residual nitrite (Woolford and Cassens, 1977) or blocking the reaction between nitrite and amines (Mirvish et al., 1972). However, bacon still appears to retain a potential, though reduced nitrosamine hazard. Therefore, various approaches are being considered to reduce input and/or residual nitrite and the potential nitrosamine formation (Sebranek, 1979; Sofos and Busta, 1980).

Irradiation has been proposed as a possible means of reducing levels of sodium nitrite used to control *C. botulinum* in cured meats (Rowley and Brynjolfsson, 1980). The calculated "12D" dose (dose reducing the number of viable spores of *C. botulinum* by 12 $\log_{10}$s) required for the sterilization at −30 ± 10°C of ham, cured with 25 μg $NaNO_2$/g and 100 μg $NaNO_3$/g and bacon, cured with 156 μg $NaNO_2$/g and 500 μg $NaNO_3$/g, at an irradiation temperature of 1.7–10°C was 3.1 (Anellis et al., 1977) and 2.65–2.87 Mrad (Anellis et al., 1965), respectively. Since irradiation could be used to inactivate *C. botulinum* spores in cured meats, research was initiated in the seventies to reduce added nitrite in corned beef (Shults et al., 1977). Research was extended to bacon to reduce the amount of added nitrite from the commercially used level of 120 μg/g to a lower level without sacrificing quality. Initially, with vacuum packed raw and prefried bacon in metal cans or aluminum laminated pouches and irradiated with sterilizing doses at either 5°C or −30 ± 10°C, it was shown that good quality bacon could be obtained when the nitrite was either reduced to 20 μg/g or eliminated (Wierbicki, 1979). Bacon without added nitrite turned reddish brown during frying. However, other quality characteristics (odor, flavor and texture) were comparable to those of the nitrite cured bacon (Wierbicki, 1979). Irradiation (3.0 Mrad at −40°C) reduced residual nitrite in bacon and destroyed preformed volatile nitrosamines that were present in the bacon prior to irradiation (Fiddler et al., 1981). Raw bacon (no nitrite or 20 μg/g) vacuum packed (one pound) in commercial films, irradiated and stored under refrigeration was of acceptable quality (Wierbicki and Heiligman, 1980). Furthermore, the irradiated bacon was free of adverse oxidative changes.

In the present study, the nitrite concentration was increased from 20 to 40 μg/g to assure good color development in bacon when pumped under industrial conditions. The objectives of this study were to determine the effects of low dose irradiation (≤ 1.5 Mrad) on the spoilage of uninoculated "low sugar" bacon, cured with 40 μg $NaNO_2$/g or without nitrite, at 5 and 27°C and on *C. botulinum* toxin formation in inoculated bacon temperature abused at 27°C.

MATERIALS & METHODS

Bacon preparation

Three lots of bacon samples were prepared in a USDA inspected bacon plant. Thorough control was exercised to assure that each lot of bacon was kept in separate stainless steel containers, that there was no contamination of the processing equipment by pickles containing different levels of nitrite, that the potable water used was free of nitrate and nitrite, and that the cured bellies of each lot were hung far enough apart in the smokehouse to minimize cross contamination with nitrite.

Fresh, uncut bacon bellies (11.5 ± 0.5 lb/belly) from 24 butcher hogs, were subdivided 1 day after slaughter into three lots. Each lot, containing left and right bellies from different hogs, was stored at 2°C for 3 days until equipment became available for curing. Each belly was injector-pumped to 12.5% added weight with curing ingredients dissolved in potable water, then stored overnight in a 2°C cooler to allow diffusion of the added pickle within the bellies. The drained individual bellies were weighed to determine actual concentrations of pickle prior to smoking. Based on our prior experience, the 12.5% added pickle results in 11.0% retention based on initial weight of the fresh bellies. on this basis, the concentration of curing components in the pickles was calculated to yield the following additions to the processed bacon: 1.5% sodium chloride, 0.25% sucrose, 0.3% sodium tripolyphosphates, 0.055% sodium erythorbate and 0, 40 or 120 μg $NaNO_2$/g. Pumped bellies were stored overnight (18 hr) in 2°C cooler prior to processing in a commercial

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smokehouse, which took 6 hr and 35 min to reduce the weight of the bellies to 100% yield-to-raw of the original weight as required by the USDA regulations, and to reach 53°C internal temperature in the thickest parts of the bellies. Hickory sawdust smoke was applied for 4 hr at a smokehouse temperature of 51°C followed by processing for 2 hr and 35 min without smoke at 57°C until reaching the objectives specified above. The smoked bellies were stored at −18°C for 18 hr to speed up the chilling process, then tempered for 22 hr at 2°C to cool down the smoked bellies to the slicing temperature of ca −1.5°C, according to commercial practice. Bacon slices, 1.5 mm thick, were cut and within 3 hr after slicing the bacon was delivered to the U.S. Army Natick R&D Laboratories. At the time of delivery the product temperature was 4°C ± 1°C; this temperature was maintained during the preparation of samples for inoculation. Slices of bacon were packed so that each package for inoculation contained five randomized slices (ca 120g); one slice taken from each of five bellies with the same curing ingredients. Representative slices from each lot were removed during packaging and used for determination of the proximate composition (Table 1) by standard procedures (AOAC, 1980).

Test organism

Spore crops of five type A (33A, 36A, 62A, 77A, 12885A) and five type B (9B, 40B, 41B, 53B, 67B) strains of *C. botulinum* were produced using 2% $(NH_4)_2SO_4$ as the liquid phase of a biphasic system (Anellis et al., 1972) and stored at 4–5°C. Spore concentrations were determined as previously described (Anellis et al., 1975).

Bacteriological examination of uninoculated bacon

Uninoculated vacuum packaged samples (nonirradiated and irradiated) were incubated at 27°C or 5°C for a maximum of 61 days. Periodically, samples were checked for sour (acid) off-odors and for numbers and types of microorganisms. Twenty-five grams of bacon from each of two packages were introduced into a Waring Blendor jar with 450 ml of Butterfield's buffered water (Speck, 1976), and blended for 2 min at ambient temperature. Further dilutions were made in Butterfield's buffered water. Assays for aerobic mesophiles, yeast and mold, enterococci, lactobacilli, staphylococci, coliforms and *E. coli* were carried out, as described in *Compendium of Methods for the Microbiological Examination of Foods* (Speck, 1976). Lactobacillus agar and brilliant green lactose bile broth were obtained from Baltimore Biological Laboratory, Cockeysville, MD. All other media (plate count agar, potato dextrose agar, KF steptococcal agar, Baird-Parker agar, lauryl sulfate tryptose and EC broths) were purchased from Difco Laboratories, Detroit, MI.

Inoculation of bacon in a biohazard laminar flow hood

On the day of inoculation, spores of each strain were heat-shocked at 80°C for 10 min, cooled rapidly in ice water, decimally diluted in chilled, sterile distilled water and mixed in equal numbers to yield an inoculum of either 200 or 20,000 spores/0.3 ml. The aqueous spore inoculum was sampled for enumeration before inoculation of the bacon and again after half and all of the bacon had been inoculated. Changes in numbers of spores were within experimental error. During inoculation the spore suspension was kept cold. Spore suspension (0.3 ml; six–0.05 ml drops) was added to five slices of bacon by automatic syringe (Filamatic vial filler, Model AB, National Instrument Company, Inc., Baltimore, MD) and spread with a sterile bent glass rod. The five bacon strips were folded end to end, placed in Saran-coated polyester pouches (O_2 permeability of 9–15 cc/mil/m^2/24 hr at 23°C), vacuum sealed, packed 10 per cardboard carton (42 x 37 x 3 cm) and stored at 4–5°C for about 24 hr prior to irradiation.

Table 1—Proximate chemical analysis of unirradiated bacon

Added $NaNO_2$ (μg/g)	Constituents[a]						
	Protein	Fat	H_2O	NaCl	p[b]	Ash	pH
	%						
0	10.74	47.71	38.47	1.80	0.171	2.57	6.0
40	10.66	49.95	36.73	1.55	0.157	2.28	6.0
120	8.52	55.78	32.76	1.49	0.138	2.13	6.1

[a] Average of duplicate determinations of a composite sample. The latter contained 9 slices (3 slices from each end and the center) per belly. For bacon with 0, 40 and 120 μg $NaNO_2$/g there were a total of 21, 27 and 8 bellies, respectively.
[b] As sodium tripolyphosphate.

Irradiation

Lots of 20 pouches per nitrite level (0 and 40 μg/g) were irradiated (0.0, 0.5, 1.0, 1.5 Mrad) with an electron accelerator (10 MEV) at 5 ± 6°C. Pouches were packed 10 per carton with minimum overlap. Each carton contained five pouches of bacon with no added nitrite and five pouches of bacon with 40 μg of $NaNO_2$/g. Bacon containing 120 μg of $NaNO_2$/g was not irradiated.

Monitoring gas production

All inoculated pouches were incubated at an abuse temperature of 27 ± 0.5°C for a maximum of 60 days. They were examined daily for swelling (separation of pouch from bacon). Any pouch which showed a loss of vacuum was discarded.

Toxin assay and determination of viable *C. botulinum* cells

Pouches that were swollen for 3 days were assayed for *C. botulinum* toxin and surviving microorganisms. All unswollen pouches were similarly examined after the 60 day incubation period. The pouches were washed with Septisol (Vestal Lab. Div. of W.R. Grace and Company, St. Louis, MO), rinsed with tap water, immersed in 70% ethanol for 5 min and rinsed twice in sterile water. The sanitized pouch was aseptically opened under the biological hood and after 200 ml of gelatin phosphate (0.2% gelatin + 0.4% Na_2HPO_4; pH 6.2) were added, the pouch, with contents, was inserted into a double stomacher bag (18 cm x 30 cm) and the bacon homogenized with the Colworth Stomacher 400 (Cooke Laboratory Products, Div. Dynatech Labs, Inc., Alexandria, VA), for 15–30 sec. A 25 ml sample of homogenate was taken from the pouch and stored at 5–8°C as a reserve. To detect the presence of *C. botulinum* toxin, 30 ml of homogenate were centrifuged in the cold (4°C) at 2,500 x *g* for 20 min, and one white male mouse (strain CD-1, 15–18g) per sample was injected intraperitoneally with 0.5 ml of supernatant fluid. The mice were observed for 4 days. Toxic samples were confirmed by injecting 10 more mice per sample. Two of the mice were injected with the supernatant fluid; two with boiled (10 min) supernatant fluid; two with 0.75 ml of a mixture of 0.5 ml supernatant fluid and 0.25 ml of antitoxin A; two with a mixture of supernatant liquid and antitoxin B; and two with 0.75 ml of a mixture of 0.5 ml supernatant fluid and 0.5 ml of a mixture of equal volumes of antitoxins A and B.

The detection of viable *C. botulinum* cells was done as previously described (Anellis et al., 1975). Ten ml of bacon homogenate were inoculated into each of two 60 ml screw-capped bottles containing 40 ml of air-exhausted TYT broth (5% BBL thiotone, 0.5% BBL yeast extract, 0.5% BBL trypticase, 0.05% sodium thioglycolate) supplemented with 0.5% glucose and 0.6 ml of 5% $NaHCO_3$. One bottle was heated at 80°C for 10 min and rapidly cooled to about 30°C, whereas the second bottle was unheated. Both bottles were incubated at 30 ± 2°C for a maximum of 30 days. Heat-shocked bottles which showed turbidity were confirmed for *C. botulinum* by the mouse toxicity test. Unheated bottles, showing turbidity, were confirmed for *C. botulinum* only if the heat-shocked bottles were not turbid. To minimize detection of toxin possibly carried over from the original sample the turbid sample was diluted at least 1:10 prior to testing for toxin.

RESULTS

Effect of nitrite and *C. botulinum* spore level on gas and toxin production in unirradiated bacon

The time to detection of swollen and toxic pouches incubated at 27°C was dependent on both the level of nitrite and spore inoculum. Toxic pouches of bacon with 0, 40 and 120 μg $NaNO_2$/g of bacon inoculated with about two spores/g were first observed at 8, 9 and 9 days, respectively (Fig. 1A). The pouches were first observed to be swollen on 6, 7 and 7 days, respectively. They may have been toxic at this time. However, since we did not want to

miss the production of toxin, the experimental protocol was designed to test for toxin after the pouches had been swollen for 3 days. Although eventually all of the pouches of bacon with 0 and 40 μg $NaNO_2$/g swelled and became toxic (Fig. 1A, Table 2), 40 μg $NaNO_2$/g caused a definite delay in swelling and toxin formation. There was no major difference between bacon with 40 or 120 μg $NaNO_2$/g in the initiation time or extent of toxic swells through 27 days at 27°C. Although all pouches of bacon with 120 μg $NaNO_2$/g swelled only about 73% (11/15) were toxic (Fig. 1A, Table 2).

The first toxic pouches of 0, 40, and 120 μg/g of bacon inoculated with 160 spores/g were observed at 6, 6 and 9 days, respectively (Fig. 1B). All pouches stored at 27°C swelled (Table 3) and about 89, 100 and 66%, respectively, of the swollen pouches were toxic (Fig. 1B, Table 3).

Effect of irradiation and *C. botulinum* spore level on gas and toxin production in bacon with different levels of $NaNO_2$

All unirradiated pouches of bacon without nitrite and inoculated with ca 2 spores/g swelled within 12 days at 27°C and were subsequently shown to be toxic (Fig. 2A, Table 2). Although irradiation with 0.5 Mrad delayed the initiation of swelling, it did not alter the total number of pouches becoming toxic. Increasing the irradiation dose increased the lag in initiation of swelling, and the total number of toxic pouches decreased (Fig. 2A, Table 2). Irradiation with 1.5 Mrad prevented the occurrence of any swollen and/or toxic pouches during the 60 days at 27°C (Fig. 2A, Tables 2 and 4). An increase in the inoculum level to 160 spores/g (Fig. 2B) decreased the delay in swelling of unirradiated samples. Although the increased inoculum level had no major effect on samples irradiated with 0.5 Mrad, it did decrease the delay in swelling and increased the number of swollen and toxic pouches of those samples irradiated with 1.0 and 1.5 Mrad (Fig. 2B, Table 3). Only 5.0% of the pouches containing 0 μg $NaNO_2$ of bacon inoculated with 160 spores/g and irradiated with 1.5 Mrad were toxic swells after 60 days at 27°C (Table 3) compared to about 67% toxic swells of unirradiated bacon (120 μg $NaNO_2$/g) in 10 days at 27°C (Fig. 1B). Although all swollen pouches were not toxic, they did have viable *C. botulinum* cells (Tables 2 and 3).

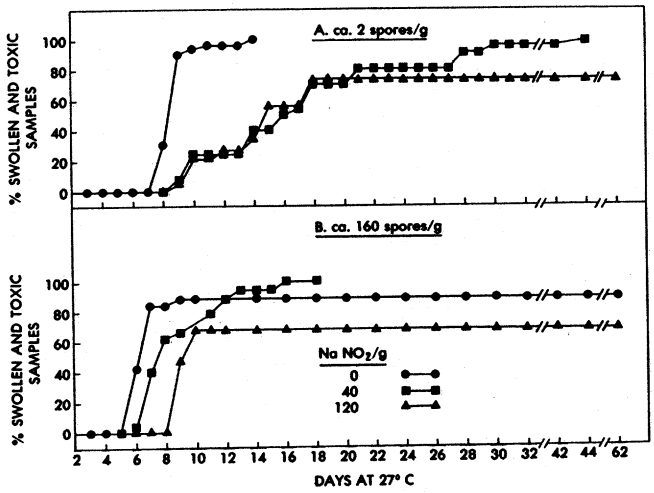

Fig. 1—Effect of concentration of nitrite and of C. botulinum spore inoculum level on the swelling of pouches of unirradiated bacon and toxin production when incubated at 27° C. All packages had been swollen for 3 days when tested for toxin.

The effect of irradiation on gas, toxin production and recoverable *C. botulinum* cells in bacon with 40 μg $NaNO_2$/g (Fig. 3A, 3B and Tables 2 and 3) was basically the same as in bacon with 0μg $NaNO_2$/g (Fig. 2A, 2B, and Tables 2 and 3). In samples inoculated with 2 spores/g, it did appear that 1.0 Mrad was more effective in preventing swollen and toxic pouches in the presence of 40 μg $NaNO_2$/g than in 0 μg $NaNO_2$/g (Table 2). This was not apparent with the higher inoculum level (Table 3).

In both irradiated and unirradiated bacon, when toxin was present, type A occurred more frequently than type B (Tables 2 and 3). Since there was no major difference in radiation resistance between type A and B spores, it may be that type A spores germinated and multiplied in bacon at a faster rate than type B spores, so that when the pouches were removed for toxin analyses, type B cells had not had the necessary time to produce toxin. In unirradiated bacon with 120 μg $NaNO_2$/g, nine of eleven toxic pouches contained both A and B toxin. None of the toxic samples contained only type B toxin.

With few exceptions, about the same number of swollen pouches of both unirradiated and irradiated bacon contained type A viable cells as contained type B cells (Tables 2 and 3). This is as expected, since the spore inoculum was

Table 2—Effect of irradiation (5 ± 6° C) and nitrite level of bacon with an inoculum of 2 spores/g on gas and toxin production and recoverable C. botulinum[a]

Radiation dose (Mrad)	Added nitrite (μg/g)	Number of pouches		Number of swollen pouches[c] with *C. botulinum* Toxin			Viable *C. botulinum*					
							Heated			Unheated		
		Tested[b]	Swollen	A	B	A and B	A	B	A and B	A	B	A and B
0.0	0	20	20	8	0	12	3	1	14	0	1	1
0.5	0	20	20	19	0	1	6	4	9	0	1	0
1.0	0	18	8	7	0	1	1	3	1	2	0	1
1.5	0	20	0	—[d]	—	—	—	—	—	—	—	—
0.0	40	20	20	10	0	10	5	5	8	0	0	2
0.5	40	18	15	15	0	0	5	4	4	0	1	1
1.0	40	16	1	0	0	0	0	0	0	0	0	1
1.5	40	18	0	—	—	—	—	—	—	—	—	—
0.0	120	15	15	2	0	9	6	1	8	0	0	0

[a] Bacon (5 slices of ca 120g) was inoculated with a mixture of spores of 10 C. botulinum strains (ca 2 spores/g of bacon), vacuum sealed, irradiated and incubated at 27 ± 0.5° C until swelling or for 60 days, whichever came first.

[b] Twenty replicate pouches were prepared per variable. However, some lost vacuum within the first 24 hr of incubation and were discarded.

[c] Pouches swollen at 27° C for 3 days were assayed for toxin, spores and vegetative cells.

[d] There were no swollen pouches to test for C. botulinum toxin or cells.

designed to contain types A and B spores of similar radiation resistance (Anellis and Koch, 1962). Only in the case of bacon containing 40 μg $NaNO_2$/g, inoculated with 160 spores/g and irradiated with either 0.5 or 1.0 Mrad did more of the swollen pouches contain viable type A cells than contained type B cells.

C. botulinum toxin and/or viable cells in unswollen samples

Four of the 99 inoculated and irradiated packages which remained unswollen during 60 days of incubation at 27°C did contain toxin (Table 4). The majority (73) of the 99 unswollen samples contained recoverable *C. botulinum* cells whether toxin was produced or not.

Spoilage of uninoculated nonirradiated bacon

The uninoculated, nonirradiated bacon had an indigenous microflora of about 1.0–2.7 x 10^3 aerobic mesophiles (APC) and 2.0–5.5 x 10^2 yeasts per gram (Tables 5 and 6). Through 5, 8 and 8 days of storage at 5°C, yeasts were the predominant microflora for bacon with 0, 40 and 120 μg $NaNO_2$/g (Table 5). At 5°C, the sour (acid) off-odor of uninoculated, nonirradiated bacon containing 0 and 40 μg $NaNO_2$/g was first observed on days 35 and 61, respectively. There was no evidence throughout the 61 days of an off-odor of bacon containing 120 μg of $NaNO_2$/g. At all three nitrite levels (0, 40 and 120 μg/g), *Staphylococcus aureus* and *Escherichia coli* were not detected and lactobacilli, coliforms, and enterococci were either not detected or were present only in very small numbers during the incubation period.

At 27°C, bacon containing 0 and 40 μg of $NaNO_2$ was first observed to have a sour (acid) odor on the eighth day (Table 6), whereas bacon containing 120 μg of $NaNO_2$/g did not have an off-odor until the thirteenth day. In contrast to storage at 5°C yeasts were never predominant in any of the bacon stored at 27°C (Table 6). At the latter storage temperature, large numbers of enterococci were present in all of the stored bacon samples. *S. aureus* and *E. coli* were not detected in any of the bacon samples examined.

Effect of irradiation on the spoilage of uninoculated bacon

Bacon containing 0 and 40 μg $NaNO_2$/g was sterilized when irradiated with 1 Mrad, as indicated by aerobic plate

Table 3—Effect of irradiation (5 ± 6° C) and nitrite level of bacon with an inoculum of 160 spores/g on gas and toxin production and recoverable C. botulinum[a]

Radiation dose (Mrad)	Added nitrite (μg/g)	Number of pouches		Number of swollen pouches[c] with								
				C. botulinum Toxin			Viable *C. botulinum*					
							Heated			Unheated		
		Tested[b]	Swollen	A	B	A and B	A	B	A and B	A	B	A and B
0.0	0	19	19	5	0	12	0	0	19	0	0	0
0.5	0	20	20	20	0	0	7	5	7	0	1	0
1.0	0	20	20	20	0	0	5	6	6	0	0	3
1.5	0	20	2	1	0	0	0	0	1	1	0	0
0.0	40	19	19	5	0	14	3	0	14	0	0	2
0.5	40	17	17	17	0	0	10	0	6	0	0	1
1.0	40	18	18	17	0	0	8	1	4	3	1	1
1.5	40	15	0	—	—	—	—	—	—	—	—	—
0.0	120	15	15	3	0	7	6	1	8	0	0	0

[a] Bacon (5 slices of ca 120g) was inoculated with a mixture of spores of 10 C. botulinum strains 160, vacuum sealed, irradiated and incubated at 27 ± 0.5°C until swelling or for 60 days, whichever came first.
[b,c] As described in Table 2.

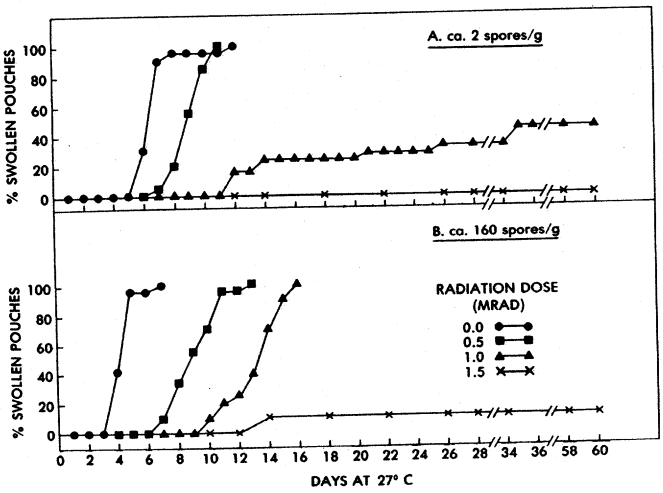

Fig. 2—Effect of irradiation at 5 ± 6°C and C. botulinum spore inoculum level on the swelling of pouches of bacon (no added nitrite) incubated at 27°C. Inoculated bacon was vacuum packed and irradiated at 5 ± 6°C.

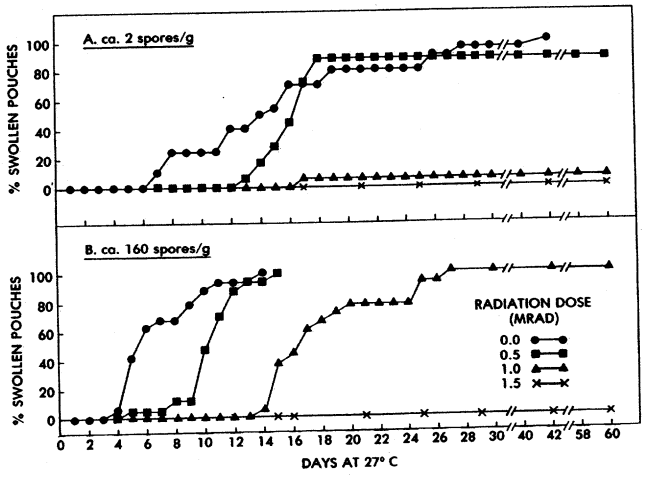

Fig. 3—Effect of irradiation at 5 ± 6°C and C. botulinum spore inoculum level on the swelling of pouches of bacon (40 μg $NaNO_2$/g) incubated at 27°C. Inoculated bacon was vacuum packed and irradiated at 5 ± 6°C.

counts (APC) of less than 1/g and absence of recoverable bacteria throughout the 60 day storage period at 27°C (data not shown). Under similar conditions of abuse, unirradiated bacon with 120 μg of added $NaNO_2$/g had spoiled by 13 days and had an APC of 2.0×10^7/g (Table 6).

An irradiation dose of 0.5 Mrad reduced the APC of bacon with 0 or 40 μg $NaNO_2$/g to <1.0/g (data not shown). At 27°C the APC of bacon with 0 and 40 μg $NaNO_2$/g increased to 2.0×10^7 and 4.0×10^3, respectively by 60 days. At this time the counts of yeasts, enterococci, staphylococci, lactobacilli and coliforms were ca 10^3, <10, <10, <10 and <3 per gram of bacon, respectively, and there was no evidence of off-odors.

DISCUSSION

THE INHIBITION of *C. botulinum* toxin formation in cured meats is generally acknowledged to be due to a combination of such factors as: brine concentration, pH, numbers of *C. botulinum* types A and B proteolytic spores, water activity, added and residual nitrite concentration, storage temperature, spore inactivation and/or injury, and product composition (Christiansen, 1980). Thus, nitrite is a part of a complex system which provides safety from botulism (Tompkin, 1978).

Surveys of the incidence of *C. botulinum* in bacon have been conducted in the United Kingdom (Roberts and Smart, 1976; 1977) and Canada (Hauschild and Hilsheimer, 1980); the number ranging from 6.4×10^{-5} *C. botulinum* cells/g in Canada to ca 10^{-3}/g in the United Kingdom. Thus even the lower spore level of 2/g, as used in our experiments, is considerably above the level expected in bacon.

In our studies with bacon inoculated with two spores/g, as the nitrite level was increased from 0 to 40 μg/g the onset of toxin development took longer, but in both cases 100% of the pouches became toxic. With the low inoculum number, there was no difference in the onset of toxic pouches and little difference in the rate of toxin formation in bacon with 40 and 120 μg of $NaNO_2$/g of bacon. Increasing the spore inoculum to 160 spores/g enhanced the onset of toxic spoilage of bacon with 0 and 40 μg $NaNO_2$/g, but not with 120 μg $NaNO_2$/g. At an abuse temperature of 27°C, the 120 μg $NaNO_2$/g was not effective in preventing toxin formation even with a low level (two spores/g) of inoculum.

The onset of swollen pouches of bacon without or with 40 μg $NaNO_2$/g was progressively delayed as the irradiation dose was increased. A significant finding was that an irradiation dose of 0.5 Mrad reduced but did not eliminate the normal microflora of bacon with 40 μg $NaNO_2$/g and delayed the onset of swollen pouches, previously inoculated with 2 spores/g, at 27°C to 13 days. Unirradiated pouches of bacon with 120 μg $NaNO_2$/g and 2 spores/g were first observed to be swollen in 6 days at 27°C. Thus a low dose of 0.5 Mrad extended the safe shelf life of temperature abused bacon with 40 μg $NaNO_2$/g by at least one week. Bacon with or without 40 μg $NaNO_2$/g, inoculated with 2 spores/g, irradiated with 1.5 Mrad and incubated at 27°C for 60 days was free of toxin, but the

Table 4—Presence of C. botulinum toxin and viable cells in some unswollen pouches of bacon inoculated with C. botulinum spores, irradiated and incubated[a]

Added nitrite (μg/g)	*C. botulinum* (spores/g)	Radiation dose (Mrad)	Number of pouches			Number of unswollen pouches[c] with *C. botulinum* Toxin			Viable *C. botulinum*					
									Heated			Unheated		
			Tested[b]	Swollen	Unswollen	A	B	A and B	A	B	A and B	A	B	A and B
0	2	1.0	18	8	10	1	0	0	1	1	0	1	0	2
0	2	1.5	20	0	20	0	0	0	1	12	2	0	0	0
0	160	1.5	20	2	18	0	0	0	1	8	1	2	2	1
40	2	0.5	18	15	3	1	0	0	0	2	0	0	0	0
40	2	1.0	16	1	15	0	0	0	1	3	4	1	0	2
40	2	1.5	18	0	18	0	0	0	0	6	3	2	2	0
40	160	1.5	15	0	15	2	0	0	1	6	4	1	0	0

[a] Bacon (5 slices of ca 120g) was inoculated with a mixture of spores of 10 C. botulinum strains to give the final concentration as indicated, vacuum sealed, irradiated and incubated at 27 ± 0.5°C until swelling or for 60 days, whichever came first.

[b] As described in Table 2.

[c] Pouches unswollen after 60 days incubation at 27°C were assayed for toxin, spores and vegetative cells.

Table 5—Microbial counts of non-irradiated bacon stored at 5° C in vacuum sealed pouches

Storage time (days)	Average colony count per gram[a]								
	Aerobic mesophiles			Yeasts			Staphylococci[b]		
	$NaNO_2$ (μg/g)			$NaNO_2$ (μg/g)			($NaNO_2$ (μg/g)		
	0	40	120	0	40	120	0	40	120
0	2.7×10^3	1.0×10^3	2.3×10^3	5.5×10^2	2.0×10^2	2.0×10^2	<10	<10	<100
2	1.6×10^3	2.6×10^3	1.4×10^3	1.7×10^3	3.0×10^2	3.0×10^2	100	50	<100
5	2.4×10^4	2.9×10^3	7.0×10^2	2.0×10^4	3.0×10^3	2.0×10^3	120	<10	<100
8	1.2×10^5	1.5×10^4	7.4×10^3	5.0×10^4	4.0×10^4	2.0×10^4	220	70	<100
16	4.9×10^6	1.4×10^5	2.4×10^4	5.1×10^4	6.5×10^4	3.0×10^4	220	<10	<100
28	1.1×10^7	8.5×10^5	4.8×10^6	1.0×10^4	4.5×10^4	5.0×10^4	5.6×10^5	2.4×10^3	<100
35	1.7×10^7[c]	7.9×10^6	4.3×10^6	7.2×10^4[c]	4.2×10^4	9.0×10^4	4.7×10^5[c]	3.0×10^4	<100
51	ND[d]	1.2×10^7	7.0×10^5	ND	1.0×10^7	2.0×10^5	ND	1.7×10^5	<100
61	ND	1.1×10^7[c]	5.0×10^6	ND	5.0×10^4[c]	3.0×10^4	ND	<10^3[c]	<100

[a] Average of duplicate plate counts.

[b] Staphylococcus aureus was not detected.

[c] Samples were first observed to have a sour odor.

[d] ND, no data.

Storage time (days)	$NaNO_2$ (μg/g)	Average count per gram[a]					
		Aerobic mesophiles	Yeasts	Enterococci	Staphylococci[b]	Lactobacilli	Coliforms (MPN)[c]
0	0	2.7×10^3	5.5×10^2	<100	<10	<100	3.6
	40	1.0×10^3	2.0×10^2	<100	<10	<100	<3.0
	120	2.3×10^3	2.0×10^2	<100	<10	<100	9.1
2	0	4.2×10^7	8.7×10^4	1.4×10^6	2.0×10^5	250	$>1.1 \times 10^3$
	40	8.0×10^6	2.1×10^5	2.0×10^5	2.0×10^5	<100	$>1.1 \times 10^3$
	120	1.1×10^7	2.0×10^5	5.0×10^4	7.7×10^5	5.0×10^4	$>1.1 \times 10^3$
5	0	2.0×10^8	ND[d]	1.6×10^7	3.5×10^6	2.1×10^3	$>1.1 \times 10^6$
	40	9.0×10^7	ND	6.0×10^6	7.5×10^5	2.6×10^3	4.6×10^5
	120	4.0×10^7	ND	3.0×10^6	9.3×10^5	6.0×10^3	4.6×10^5
8	0[e]	1.0×10^8	5.0×10^4	1.8×10^7	3.1×10^6	1.8×10^5	3.6×10^5
	40[e]	1.1×10^8	4.0×10^4	6.0×10^6	2.2×10^5	1.5×10^5	3.0×10^4
	120	1.4×10^7	2.0×10^4	6.0×10^5	3.0×10^4	1.0×10^5	$<4.6 \times 10^5$
13	0	ND	ND	ND	ND	ND	ND
	40	ND	ND	ND	ND	ND	ND
	120[e]	2.0×10^7	5.0×10^3	3.0×10^6	1.2×10^4	6.4×10^6	4.6×10^4

[a] Except for coliforms the average count was of duplicate plate counts.
[b] Staphylococcus aureus was not detected.
[c] Three-tube most probable number; Escherichia coli was not detected.
[d] ND, no data.
[e] Samples were first observed to have a sour odor.

majority of unswollen pouches (15/20; 13/18) contained recoverable *C. botulinum* cells. Thus, the absence of toxic pouches was likely due to both spore inactivation and injury; the injured spores were unable to develop and produce toxin in the bacon. Various investigators (Anellis et al., 1965; Greenberg et al., 1965) have shown that *C. botulinum* spores surviving irradiation were not capable of producing toxin in cured meats. Studies with model systems showed that irradiation sensitized *Clostridium sporogenes* and *C. oedematiens* (Roberts et al., 1965) and *C. botulinum* 62 A spores (Rowley et al., 1970) to sodium chloride.

In addition to controlling higher-than-expected levels of *C. botulinum* spores in bacon, a dose of 1.5 Mrad would inactivate about 10^{14} *Salmonella enteritidis, Staphylococcus aureus* and *Escherichia coli* cells, assuming the D values as reported by Anellis et al. (1979). A dose of 1 Mrad eliminated the normal flora in our study and enabled bacon without or with added nitrite (40 μg/g) to be kept at 27°C for 60 days without any evidence of microbial spoilage. Uninoculated bacon with 120 μg of added $NaNO_2$/g had a sour odor within 8–13 days at 27°C. However, if maintained at 5°C, the latter bacon had no sour odor through 61 days. This emphasizes the importance of refrigeration for unirradiated bacon with 120 μg $NaNO_2$/g.

The safety against botulism provided by nitrite, is significant but not absolute. Nitrite may be depleted during storage (Christiansen et al., 1978; Tompkin et al., 1978) or it may be overcome if the *C. botulinum* spore level is high (Christiansen et al., 1973; Christiansen et al., 1974; Sofos et al., 1979). Similarly, the protection provided by 1.5 Mrad was overcome by increasing the spore level from 2–160 spores/g. Under these conditions two of 20 tested pouches of bacon with no added nitrite were swollen and one contained toxin. In the case of bacon with 40 μg $NaNO_2$/g, although none of the 15 tested pouches was swollen, two contained toxin. Others have observed unswollen toxic pouches of inoculated, but unirradiated bacon (Anellis et al., 1965; Sofos et al., 1980). Some of the samples of bacon containing either 0 or 40 μg $NaNO_2$/g and 0.26% potassium sorbate did not show gas, but were toxic after 60 days at 27°C (Sofos et al., 1980). The presence of unswollen toxic pouches of bacon observed in this and other studies requires further experimentation before its significance can be assessed.

It is evident from these studies that an irradiation dose of 0.5 Mrad delayed the onset of swollen and toxic pouches of temperature abused bacon, with 40 μg $NaNO_2$/g and a higher than normally expected level (2/g) of *C. botulinum* spores, by about one more week than observed for unirradiated control bacon (120 μg $NaNO_2$/g). Furthermore, although this low dose of irradiation did not eliminate the normal microflora it would reduce the numbers of foodborne pathogens, such as salmonellae and *S. aureus* 10^4 to 10^5-fold. Under similar conditions an irradiation dose of 1.5 Mrad completely inactivated the normal microflora and left viable but injured *C. botulinum* spores that were unable to produce toxin when incubated at 27°C for 60 days.

REFERENCES

Anellis, A., Berkowitz, D., Kemper, D., and Rowley, D.B. 1972. Production of types A and B spores of Clostridium botulinum by the biphasic method: Effect on spore population, radiation resistance and toxigenicity. Appl. Microbiol. 23: 734.

Anellis, A., Grecz, N., Huber, D.A. Berkowitz, D., Schneider, M.D. and Simon, M. 1965. Radiation sterilization of bacon for military feeding. Appl. Microbiol. 13: 37.

Anellis, A. and Koch, R.B. 1962. Comparative resistance of strains of Clostridium botulinum to gamma rays. Appl. Microbiol. 10: 326.

Anellis, A., Rowley, D.B. and Ross, E.W. Jr. 1979. Microbiological safety of radappertized beef. J. Food Protect. 42: 927.

Anellis, A., Shattuck, E., Latt, T., Songpasertchal, S., Rowley, D.B., and Ross, E.W. Jr. 1977. Gamma irradiation at −30 ± 10°C of low level nitrite/nitrate ham. In "Spore Research 1976," Vol. 2, (Ed.) Barker, A.N., Wolf, J., Ellar, D.J., Dring, G.J., and Gould, G.W., p. 631. Academic Press, London.

Anellis, A., Shattuck, E., Rowley, D.B., Ross, E.W. Jr., Whaley, D.N., and Dowell, V.R. Jr. 1975. Low-temperature irradiation of beef and methods for evaluation of a radappertization process. Appl. Microbiol. 30: 811.

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Cassens, R.B., Greaser, M.L., Ito, T., and Lee, M. 1979. Reactions of nitrite in meat. Food Technol. 33(7): 46.

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. J. Food Sci. 35: 668.

Christiansen, L.N. 1980. Factors influencing botulinal inhibition by nitrite. Food Technol. 34(5): 237.

Christiansen, L.N., Johnston, R.W., Kautter, D.A., Howard, J.W., and Aunan, W.J. 1973. Effect of nitrite and nitrate on toxin production by Clostridium botulinum and on nitrosamine formation in perishable canned comminuted cured meat. Appl. Microbiol. 25: 357 (Erratum 26: 653).

Christiansen, L.N., Tompkin, R.B., and Shaparis, A.B. 1978. Fate of Clostridium botulinum in perishable canned cured meat at abuse temperature. J. Food Protect. 41: 354.

Christiansen, L.N., Tompkin, R.B., Shaparis, A.B., Kueper, T.V., Johnston, R.W., Kautter, D.A., and Kolari, O.J. 1974. Effect of sodium nitrite on toxin production by Clostridium botulinum in bacon. Appl. Microbiol. 27: 733.

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Crosby, N.T. and Sawyer, R. 1976. N-nitrosamines: A review of chemical and biological properties and their estimation in foodstuffs. Adv. Food Res. 22: 1.
Fiddler, W., Gates, R.A., Pensabene, J.W., Phillips, J.G., and Wierbicki, E. 1981. Investigations on nitrosamines in irradiation-sterilized bacon. J. Agri. Food Chem. 29: 551.
Gray, J.I. and Randall, C.J. 1979. The nitrite/N-nitrosamine problem in meats. An update. J. Food Protect. 42: 168.
Greenberg, R.A., Bladel, B.O., and Zingelmann, W.J. 1965. Radiation injury of Clostridium botulinum spores in cured meat. Appl. Microbiol. 13: 743.
Hauschild, A.H.W. and Hilsheimer, R. 1980. Incidence of Clostridium botulinum in commercial bacon. J. Food Protect. 43: 564.
Mirvish, S.S., Wallcave, L., Eagen, M., and Shubik, P. 1972. Ascorbate-nitrite reaction: Possible means of blocking the formation of carcinogenic N-nitroso compounds. Science 177: 65.
Pivnick, H., Johnston, M.A., Thacker, C., and Loynes, R. 1970. Effect of nitrite on destruction and germination of Clostridium botulinum and putrefactive anaerobes 3679 and 3679h in meat buffer. Can. Inst. Food Sci. Technol. J. 3: 103.
Preussman, F. 1974. Toxicity of nitrite and N-nitroso compounds. In "Proc. International Symp. on Nitrite in Meat Products," (Ed.) Krol, B. and Tinbergen, B.J., p. 217. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
Roberts, T.A., Ditchett, P.J., and Ingram, M. 1965. The effect of sodium chloride on radiation resistance and recovery of irradiated anaerobic spores. J. Appl. Bacteriol. 28: 336.
Roberts, T.A. and Smart, J.L. 1976. The occurrence and growth of Clostridium ssp. in vacuum-packed bacon with particular reference to Cl. perfringens (welchii) and Cl. botulinum. J. Food Technol. 11: 229.
Roberts, T.A. and Smart, J.L. 1977. The occurrence of clostridia, particularly Clostridium botulinum, in bacon and pork. In "Spore Research 1976," (Ed.) Barker, A.N., Wolf, J., Ellar, D.J., Dring, G.J., and Gould, G.W., Vol. 2, p. 911. Academic Press, New York.
Rowley, D.B. and Brynjolfsson, A. 1980. Potential uses of irradiation in the processing of food. Food Technol. 34(10): 75.
Rowley, D.B., El-Bisi, H.M., Anellis, A., and Snyder, O.P. 1970. Resistance of Clostridium botulinum to ionizing radiation as related to radappertization of foods. In "Proc. First U.S.-Japan Conf. on Toxic Microorganisms, Honolulu, HI, 1968," (Ed.) Herzberg, M., p. 459. UJNR Joint Panels on Toxic Microorganisms and U.S. Dept. of the Interior, Washington, DC.
Sander, J. 1974. Formation of N-nitroso compounds in laboratory animals. A short review. In "Proc. of International Symposium on Nitrite in Meat Products," (Ed.) Krol, B. and Tinbergen, B.J., p. 243. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
Sebranek, J.G. 1979. Advances in the technology of nitrite use and consideration of alternatives. Food Technol. 33(7): 58.
Shults, G.W., Cohen, J.S., Howker, J.J., and Wierbicki, E. 1977. Effects of sodium nitrate and sodium nitrite additions and irradiation processing variables on the color and acceptability of corned beef briskets. J. Food Sci. 42: 1506.
Sofos, J.N. and Busta, F.F. 1980. Alternatives to the use of nitrite as an antibotulinal agent. Food Technol. 34(5): 244.
Sofos, J.N., Busta, F.F., and Allen, C.E. 1979. Effects of sodium nitrite on Clostridium botulinum toxin production in frankfurter emulsions formulated with meat and soy proteins. J. Food Sci. 44: 1267.
Sofos, J.N., Busta, F.F., Bhothipaksa, K., Allen, C.E., Robach, M.C., and Paquette, M.W. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on Clostridium botulinum toxin production in commercially prepared bacon. J. Food Sci. 45: 1285.
Speck, M.L. 1976. "Compendium of Methods for the Microbiological Examination of Foods." Amer. Public Health Assoc., Washington, DC.
Tompkin, R.B. 1978. The role and mecanisms of the inhibition of C. botulinum by nitrite—Is a replacement available? Proc. of 31st Ann. Reciprocal Conf. of Amer. Meat Sci. Assoc., 18—22 June, Univ. of Connecticut, Storrs, CT.
Tompkin, R.B., CHristiansen, L.N., and Shaparis, A.B. 1978. Effect of prior refrigeration on botulinal outgrowth in perishable canned cured meat when temperature abused. Appl. Environ. Microbiol. 35: 863.
Wasserman, A.E. and Talley, F. 1972. The effect of sodium nitrite on the flavor of frankfurters. J. Food Sci. 37: 536.
Wierbicki, E. 1979. The importance and feasibility of irradiated low nitrite meat products. Activities Report of R&D Associates 31(2): 70.
Wierbicki, E. and Heiligman, F. 1980. Irradiated bacon without and with reduced addition of nitrite. In "Proc. 26th European Meeting of Meat Research Workers," Colorado Springs, CO, 31 Aug—5 Sep 1980: 198.
Woolford, G. and Cassens, R.G. 1977. The fate of sodium nitrite in bacon. J. Food Sci. 42: 586.
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